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RESEARCH PAPER

Functional predictions of bacterial communities associated with sorghum cultivars across different rhizocompartments

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Abstract

The plant root microbiome played critical roles in nutrient acquisition and stress resilience, yet the impacts of host genotype on microbial functions remained poorly understood, particularly in economically important crops like sorghum. This study investigated how three genetically distinct sorghum cultivars (*Hongyingzi*, *Hongzhenzhu*, and *Jinza*) shaped microbial functional profiles across rhizocompartments (rhizosphere, rhizoplane, endosphere) and their contributions to soil nutrient dynamics. Using 16S rRNA sequencing combined with predictive functional profiling (PICRUSt2, FAPROTAX) and soil physicochemical analyses, we revealed compartment-specific functional stratification driven by cultivars. Results demonstrated pronounced genotype-dependent microbial functional adaptation: *Jinza* exhibited enhanced biofilm formation and aromatic compound utilization in the endosphere, while *Hongyingzi* prioritized broad metabolic versatility in the rhizoplane. Pathway enrichment analyses highlighted cultivar-specific strategies, with *Hongzhenzhu* showing heightened sulfur metabolism and nitrogen fixation in the rhizoplane, and *Jinza* displaying specialized nutrient transport pathways in the endosphere. Correlation networks further linked dominant taxa (e.g., Burkholderiales, Rhizobiales) to key metabolic processes, including pollutant degradation and stress response. Random forest models revealed increasing functional specialization from rhizosphere to endosphere, with microbial activities explaining 30% of total organic carbon variation and 28.7% of nitrogen availability in the endosphere. This compartmental gradient underscored host-mediated selection for microbial functions critical to nutrient cycling and plant performance. By disentangling genotype-driven functional adaptations in root microbiomes, this study advanced understanding of plant-microbe interactions beyond taxonomic composition, providing a framework for engineering microbiomes to enhance crop sustainability in diverse agroecosystems.

Keywords

edaphic factors, FAPROTAX database, KEGG Orthologs, pathway enrichment, PICRUSt2

Introduction

The plant root-associated microbiome, encompassing distinct rhizocompartments such as the rhizosphere, rhizoplane, and endosphere, plays indispensable roles in plant health, nutrient acquisition, and ecosystem functions, including carbon sequestration and phytoremediation (Bulgarelli et al. 2013; Trivedi et al. 2020). These microbial communities are shaped by dynamic interactions between plant-derived signals, soil physicochemical properties, and microbial ecological strategies (Schmidt et al. 2019; Xiong et al. 2021). Host genotype, in particular, exerts profound selective pressures on microbiome assembly, driving compartment-specific functional adaptations

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(Edwards et al. 2015; Lebeis et al. 2015). For example, in maize and rice, cultivar-specific differences in root exudates and immune responses lead to distinct microbial recruitment patterns across rhizocompartments, with implications for nitrogen fixation, stress tolerance, and organic matter decomposition (Peiffer et al. 2013; Edwards et al. 2015). However, while taxonomic shifts in root-associated microbiomes have been extensively documented, the functional stratification of microbial communities—particularly in response to plant genotype—remains underexplored. This gap limits our ability to link microbial traits to plant performance and soil nutrient dynamics, especially in economically significant crops like sorghum (*Sorghum bicolor*).

Sorghum, a staple grain crop and key ingredient in traditional Chinese liquor production (Wang et al. 2015), exhibits marked genetic diversity among cultivars (Yan et al. 2018), which may differentially influence root-associated microbiomes. For example, glutinous sorghum varieties such as *Hongyingzi* (HY), *Hongzhenzhu* (HZ), and *Jinza* (JZ) are cultivated under similar edaphic conditions but display bacterial variations in diversity of various rhizocompartment, soil nutrition contribution, and assembly mechanisms (Zhao et al. 2023). These phenotypic differences likely arise from cultivar-specific interactions with microbial communities, yet the functional mechanisms underlying these associations remain unclear. Previous work by (Zhao et al. 2023) demonstrated that sorghum rhizocompartments exhibit compartmentalized microbial diversity, driven by homogeneous selection and dispersal limitation. However, this study did not address how functional traits—such as nutrient cycling, pollutant degradation, or stress response pathways—vary across cultivars and compartments, nor how these traits correlate with soil health indicators. Resolving these questions is critical for advancing microbiome-assisted agriculture, where targeted manipulation of microbial functions could enhance crop resilience and sustainability.

Recent advances in bioinformatics tools, including PICRUSt2 and FAPROTAX, enable predictive functional inference of microbial communities using 16S rRNA amplicon data (Louca et al. 2016; Douglas et al. 2019). While these tools offer cost-effective alternatives to metagenomic sequencing, their limitations—such as reliance on reference databases and potential biases in gene inference—must be acknowledged (Matchado et al. 2024; Zhang et al. 2024). Despite these constraints, integrated applications of PICRUSt2 and FAPROTAX have yielded insights into microbial functional dynamics in diverse plant systems. For instance, (Zhang et al. 2023) identified enriched aromatic hydrocarbon degradation pathways in diseased strawberry rhizospheres, while (Zheng et al. 2024) revealed nitrogen metabolism specialization in endosymbiotic fungi. In sorghum, however, such functional analyses are scarce, particularly in the context of cultivar-specific microbiome interactions. This represents a critical knowledge gap, as functional redundancy or complementarity among microbial taxa may underlie cultivar differences in nutrient acquisition and stress adaptation.

Building on these foundations, this study investigates how three genetically distinct sorghum cultivars shape microbial functional profiles across rhizocompartments and explores linkages between these functions and soil nutrient cycling. We hypothesize that (1) microbial functional traits exhibit compartment-specific stratification influenced by plant genotype, (2) key taxa drive nutrient-related pathways in a cultivar-dependent manner, and (3) functional group contributions to edaphic factors intensify from bulk soil to the endosphere. To test these hypotheses, we combined 16S rRNA sequencing with PICRUSt2 and FAPROTAX analyses, alongside soil physicochemical measurements, to unravel cultivar-mediated functional dynamics. By focusing on early seedling stages, we aimed to capture initial host-microbe interactions that establish foundational microbial communities, though we recognize that later growth phases may reveal additional temporal shifts (Patel et al. 2015). Our approach addresses critical questions about how plant genotype fine-tunes microbial functions to optimize rhizocompartment-specific processes—a prerequisite for designing microbiome-based strategies to improve sorghum productivity in variable agroecosystems.

Materials and methods

Field experiment and sample collection

Detailed information about field experiment and sample collection could be referred to our previous work (Zhao et al. 2023), or available in Suppl. material 1: appendix S1.

Sample processing

Rhizocompartment samples—encompassing rhizosphere soil, rhizoplane soil (soil with tightly adhere to root surface), and endosphere (root tissue)—were gathered in accordance with the protocol described by Edwards et al. (2015). In summary, the acquisition and handling of samples from the rhizosphere, rhizoplane, and endosphere were conducted as follows: (i) Soil that was loosely attached to the roots was carefully dislodged by gentle shaking, and the soil layer remaining up to 2 mm on the root surface was designated as rhizosphere soil. A portion of both the rhizosphere and bulk soil samples was utilized for the assessment of physicochemical characteristics (with raw data provided in Suppl. material 1: table S1), including pH, available potassium (effK), available phosphorus (effP), available nitrogen (effN), nitrate-nitrogen (noN), ammonium-nitrogen (nhN), and total organic carbon (TOC), following the methodologies outlined by Bao (2000) and Jiao et al. (2020). The remaining rhizosphere soil was preserved at −80 °C for subsequent DNA extraction. (ii) After removing the rhizosphere soil, fresh sorghum roots were subjected to sonication for 60 seconds at a frequency of 50–60 Hz in sterile phosphate-buffered saline (PBS). The PBS solution, after filtration following the removal of roots, represented the rhizoplane compartment and was collected using sterile 0.22-μm membranes (Edwards et al. 2015; Xiong et al. 2021). (iii) Roots cleared

of the rhizosphere and rhizoplane soils underwent surface sterilization by immersion in 75% alcohol for 1 minute, followed by thorough rinsing with sterile water, and were then identified as the endospheric compartment (Edwards et al. 2015). Bulk soil and root-associated compartment samples earmarked for bacterial community analysis were stored at −80 °C to facilitate future DNA extraction. Finally, 3 cultivars (*Hongyingzi*, *Hongzhenzhu*, and *Jinza*) × 3 rhizocompartments (rhizosphere, rhizoplane, and endosphere) × 5 sample replicates + 5 bulk soil samples (as control) = 50 samples were collected.

High-Throughput Sequencing and the processing

To characterize the composition of bacterial communities, the 16S rRNA gene was subjected to high-throughput sequencing. DNA from bulk soil, rhizosphere soil, rhizoplane membrane, and root tissue was isolated with the Fast DNA® SPIN Kit (MP Biochemicals, Solon, OH, USA), ALPHA-SEQ Advanced Water DNA Kit (Magigene, Guangdong, China), and Minkagene Plant DNA Kit (Magigene, Guangdong, China), respectively. Amplification of the V3–V4 hypervariable regions was carried out with primers 319F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), as described by (Fadrosh et al. 2014). Each amplification was conducted in three technical replicates alongside no-template controls. Amplicons were combined after separation on a 2% agarose gel, and products displaying clear bands were purified with the GeneJET Gel Extraction Kit (Thermo Scientific). Purified libraries were sequenced on the Illumina MiSeq platform (300-bp paired-end mode) by Weikemo Bioinformatics Technology Co., Ltd. (Shenzhen, China).

Raw reads underwent quality control and adapter trimming with fastp (v0.14.1) and fastqc (Chen et al. 2018). Denoising and amplicon sequence variant (ASV) inference were conducted using the DADA2 pipeline integrated in QIIME2 (Callahan et al. 2016; Bolyen et al. 2019). Taxonomic assignment of ASVs was achieved by alignment against the SILVA database (v.138) (Bokulich et al. 2018). Sequences classified as chloroplast or mitochondrial origin were excluded via QIIME2's filter-seqs and filter-table modules (Bokulich et al. 2018). After rarefaction to an even depth of 14,507 reads per sample—excluding two samples that failed amplification—a total of 10,133 ASVs were retained for downstream ecological analysis.

Statistical analysis

All statistical analyses were conducted in the R environment (v4.2.2; http://www.r-project.org/) using "vegan," "clusterProfiler," "Rmisc," "KEGGprofile", "gdata", "DESeq2", "rfPermute", "reshape2", "ggsci" and "gplots" packages unless stated otherwise.

The community and phylogenetic data were input into PICRUSt2 v2.1.4 (Douglas et al. 2019) to predict bacterial involvement in metabolic pathways. Additionally, functional annotation of prokaryotic taxa was performed using the FAPROTAX database (Sansupa et al. 2021) to classify bacterial functions based on feature sequence annotation. These two predictive analyses were conducted on the Weikemo cloud platform (https://www.bioincloud.tech). To identify differentially abundant KEGG Orthologs (KOs) across various rhizocompartments (rhizosphere, rhizoplane, and endosphere) relative to bulk soil, the "DESeq2" package was employed. Enriched and depleted KOs were selected based on their False Discovery Rate (FDR, $P < 0.05$). The hypergeometric test was then used to assess the overrepresentation of each KEGG metabolic pathway (ko) among the abundantly increased KOs identified by DESeq2. A FDR-corrected P-value of < 0.05 was considered significantly overrepresented.

Spearman correlation analysis was conducted to explore relationships between overrepresented kos and dominant bacterial taxa. To identify functional predictors for edaphic factors, random forest (RF) regression models were implemented with the following parameters: 1,000 decision trees (ntree = 1000), mtry (number of variables randomly sampled at each split) set to one-third of the total predictors (default for regression tasks), and node size (nodesize) fixed at 5 to optimize model performance. Functional groups predicted by FAPROTAX served as predictors, while edaphic factors were designated as response variables. The RF models significance were evaluated using the A3 package in R, which performed 10-fold cross-validation with 1,000 permutations to assess global model validity. Variable importance was determined by the percentage increase in mean squared error (%IncMSE), with higher values indicating stronger predictive contributions (Breiman 2001). To evaluate the statistical significance of individual predictors, the rfPermute package was employed, conducting 1,000 permutations to compute empirical P-values for each functional group. Model-wide R^2 values, derived from permutation-based testing, were reported to reflect the overall explanatory power of the RF models. Predictors with $P < 0.05$ were considered statistically significant. This dual-validation framework ensured both model reliability and biological relevance of the identified functional drivers.

Results

Differentially abundant KOs and Pathway Enrichment

The PICRUSt2 resulted in a total of 7,779 KOs across all samples. Using DESeq2 for differential analysis based on the KOs abundance, we compared three sorghum cultivars (HY, HZ, JZ) against bulk soil in three rhizocompartments: rhizosphere, rhizoplane, and endosphere (Fig. 1). In the rhizosphere, JZ showed 23 increased and 4 decreased KOs, while HY exhibited 155 increased and 407 decreased KOs; HZ had no increased KOs but 2 decreased ones. The rhizoplane and endosphere displayed greater KO divergence: HZ showed the most dynamic changes in the rhizoplane

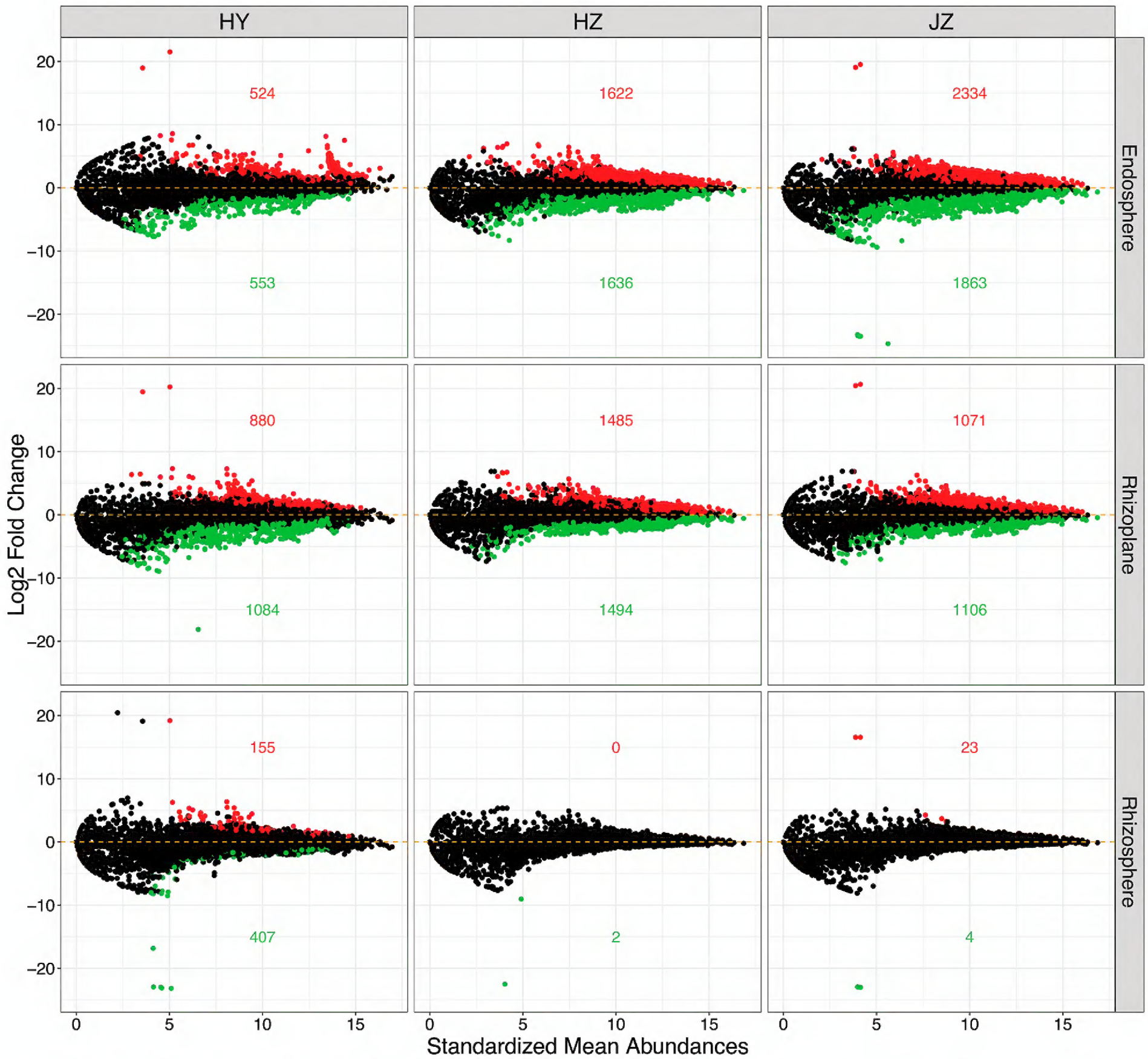


Figure 1. Comparative analysis of differentially abundant KOs across rhizocompartments in various cultivars relative to bulk soil control. Each subpanel represents a specific rhizocompartment-cultivar combination (Endosphere, Rhizoplane, Rhizosphere). Individual KO is depicted as point: red dots indicate significantly increased abundance, while green dots indicate significantly decreased abundance, with *y*-axis values showing the magnitude of change relative to bulk soil. *X*-axis denotes standardized mean abundances. Numerical labels within each subpanel specify the count of differentially increased (red) or decreased (green) KOs.

(1,485 increased/1,494 decreased KOs), followed by JZ (880/1,084) and HY (524/553). In the endosphere, JZ had the highest divergence (2,334 increased/1,863 decreased KOs), while HY showed fewer changes.

Hypergeometric enrichment analysis of abundantly increased KOs highlighted cultivar-specific metabolic strategies (Fig. 2). In the rhizosphere, HY pathways included "Bacterial secretion system" (12% gene ratio), and JZ uniquely enriched "Biofilm formation—Vibrio cholerae." The rhizoplane showed HY- dominant "Fluorobenzoate degradation" (33%) and "Flagellar assembly" (29%), HZ-dominant "Flagellar assembly" (47%), and JZ-enriched "Fluorobenzoate degradation" (39%) and "Atrazine degradation" (31%). Endosphere analyses revealed HY pathways like "Teichoic acid biosynthesis" (17%), HZ prioritizing "Bacterial chemotaxis" (77%), and JZ strongly enriching "Biofilm formation—Escherichia coli" (56%) and "ABC transporters" (43%).

Venn diagrams underscored compartment-specific adaptation: rhizoplane cultivars shared 20 core pathways, while endosphere cultivars converged on 14. Cultivar-specific adaptations included HY's 9 unique rhizosphere pathways and 6 rhizoplane functions, JZ's 13 endosphere-exclusive pathways, and HZ's 8 unique rhizoplane pathways. These patterns suggest cultivar-dependent microbial interaction strategies, with HZ and JZ exhibiting pronounced KO remodeling in rhizoplane and endosphere, respectively.

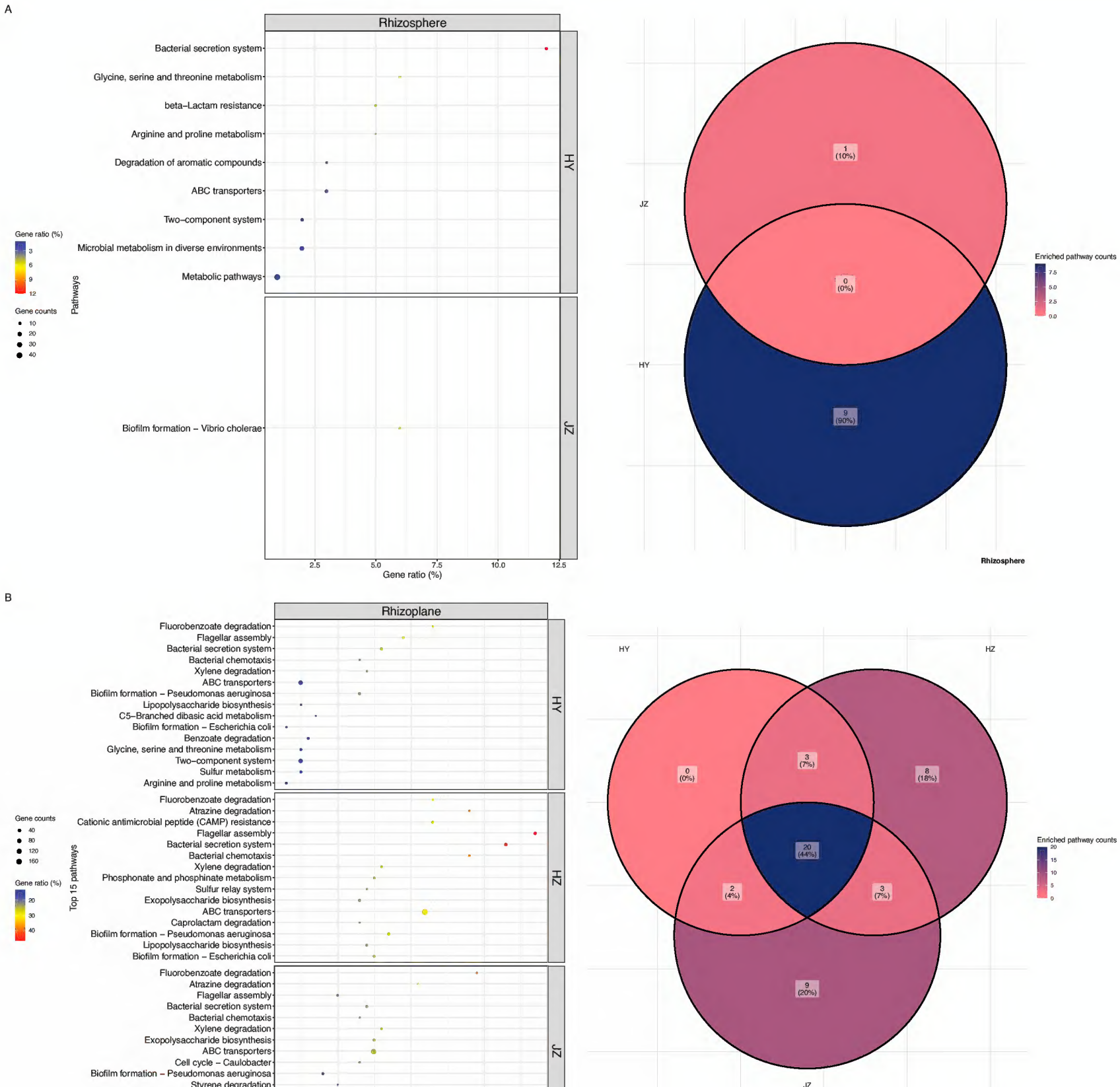


Figure 2. Pathway enrichment profiles for different cultivars across the rhizosphere (**A**), rhizoplane (**B**), and endosphere (**C**). The left panel presents a bubble plot depicting the gene ratios for each enriched pathway, while the right panel shows a Venn diagram illustrating the overlap of enriched pathways shared among the cultivars.

Community structures conveyed by dominant tax and functional groups

Relative abundance (RA) analyses of dominant bacterial taxa (order level) and functional groups (FAPROTAX predictions) were shown in Fig. 3. Taxonomic variations (Fig. 3A) included Burkholderiales dominance in HY's rhizoplane (37.91%) and HZ's endosphere (38.01%), while Rhizobiales was abundant in JZ's rhizoplane (17.18%) and HZ's endosphere (14.65%). Enterobacterales showed cultivar-specific enrichment in HZ's rhizoplane (18.74%) and JZ's endosphere (22.29%). Flavobacteriales was abundant in HY's endosphere (30.54%), and Pseudomonadales increased in HY's rhizoplane/rhizosphere. Sphingomonadales was elevated in HZ (9.34%) and JZ (6.25%). Stable taxa like Vicinamibacterales, Caulobacterales, and Xanthomonadales indicated ecological resilience across cultivars.

Functionally (Fig. 3B), "Chemoheterotrophy" was ubiquitous, with the highest levels observed in bulk soil and the rhizosphere of HZ. Aerobic chemoheterotrophy was more prominent in HZ than in JZ. Nitrogen-related functions also varied: nitrate reduction was elevated in the endosphere of JZ, while ureolysis peaked in the rhizoplane of HY. Notably, the endosphere of HY was enriched with functional groups associated with "human-associated" and "animal-associated" environments, indicating a functionally diverse microbiome. These patterns underscored how different cultivars drive the assembly of distinct bacterial communities and metabolic pathways, with HY, HZ, and JZ exhibiting unique root–microbe interaction strategies.

Associations between enriched pathways and dominant tax

Spearman correlation analysis identified compartment-specific associations between dominant bacterial taxa and enriched kos in sorghum rhizocompartments (rhizosphere, rhizoplane, endosphere) (Fig. 4). In the rhizosphere, Rhizobiales positively correlated with core metabolic pathways (e.g., glycine/serine/threonine metabolism, ko00260, $r = 0.95$; general metabolism, ko01100, $r = 0.89$), while Burkholderiales and Caulobacterales linked to bacterial environmental adaptation (ko01120, $r = 0.81$) and ABC transporters (ko02010, $r > 0.80$). Gemmatimonadales and Pyrinomonadales showed inverse correlations with these pathways (ko02010, $r = -0.86$; ko01100, $r = -0.85$). On the rhizoplane, Burkholderiales associated with aromatic compound degradation (benzoate, ko00362, $r = 0.93$; xylene, ko00622, $r = 0.93$), and Pseudomonadales correlated with exopolysaccharide biosynthesis (ko00543, $r = 0.87$) and fluorobenzoate degradation (ko00364, $r = 0.75$). Conversely, Sphingomonadales and Gemmatimonadales negatively correlated with sulfur metabolism (ko00920, $r = -0.82$) and lipopolysaccharide biosynthesis (ko00540, $r = -0.76$). Within the endosphere, Burkholderiales aligned with central carbon metabolism (ko00630, $r = 0.94$) and sulfur metabolism (ko00920, $r = 0.82$), while Rhizobiales linked to amino acid metabolism (ko00260, $r = 0.84$) and environmental adaptation (ko01120, $r = 0.72$). Negative correlations emerged for Sphingomonadales with pyruvate metabolism (ko00620, $r = -0.56$) and stress resistance (ko01503, $r = -0.44$), and for Vicinamibacterales/Pyrinomonadales with cofactor biosynthesis (ko01240, $r = -0.70$). Pseudomonadales and Streptomycetales positively associated with adaptive signaling (ko02020, $r = 0.77$) and amino acid metabolism (ko00260, $r = 0.75$), reflecting roles in host-microbe integration. These patterns underscore compartment-specific microbial contributions to nutrient cycling, stress adaptation, and ecological specialization.

Contributions of functional groups to edaphic factors

Random forest models demonstrated compartment-specific microbial functional contributions to edaphic factors, with increasing explanatory power from rhizosphere to endosphere (Fig. 5). In the rhizosphere, ureolysis showed the strongest association with total organic carbon (TOC, importance = 9.31), while aromatic compound degradation significantly influenced both TOC (8.27) and pH (12.70), collectively explaining 15.55% and 6.23% of variation in

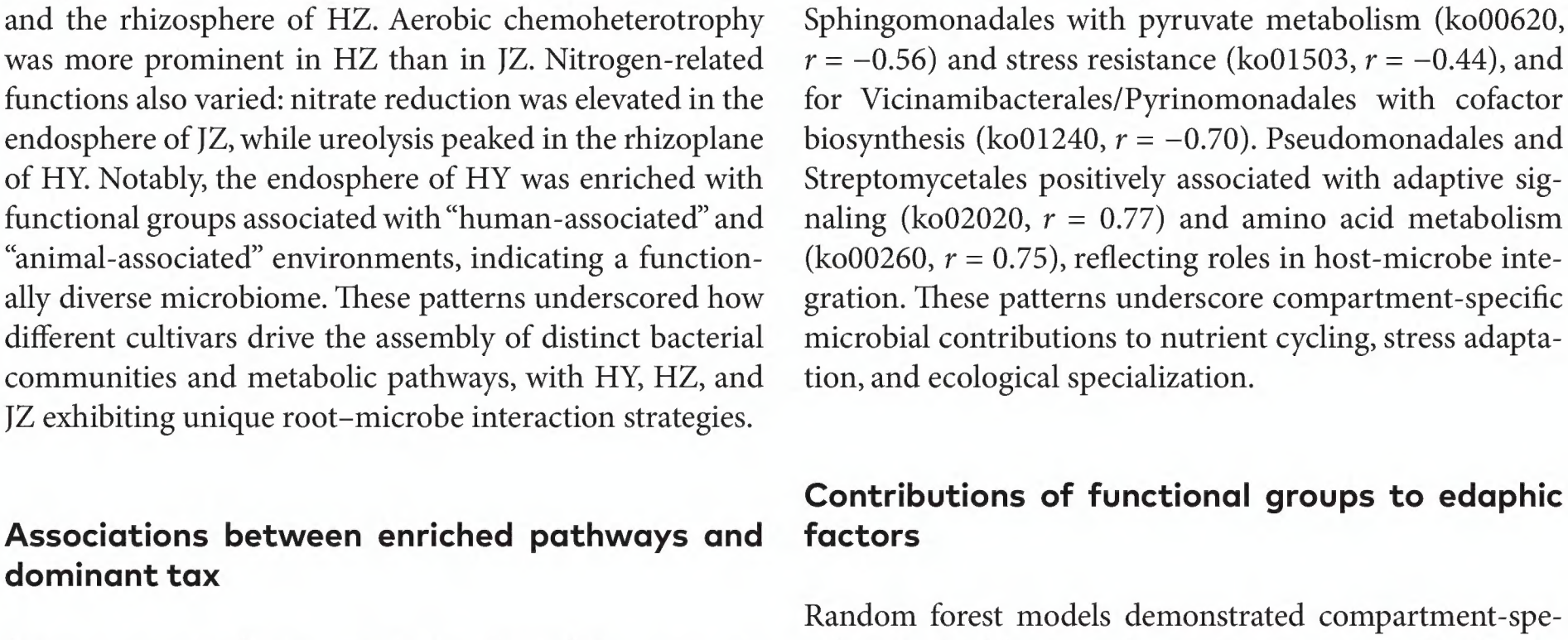
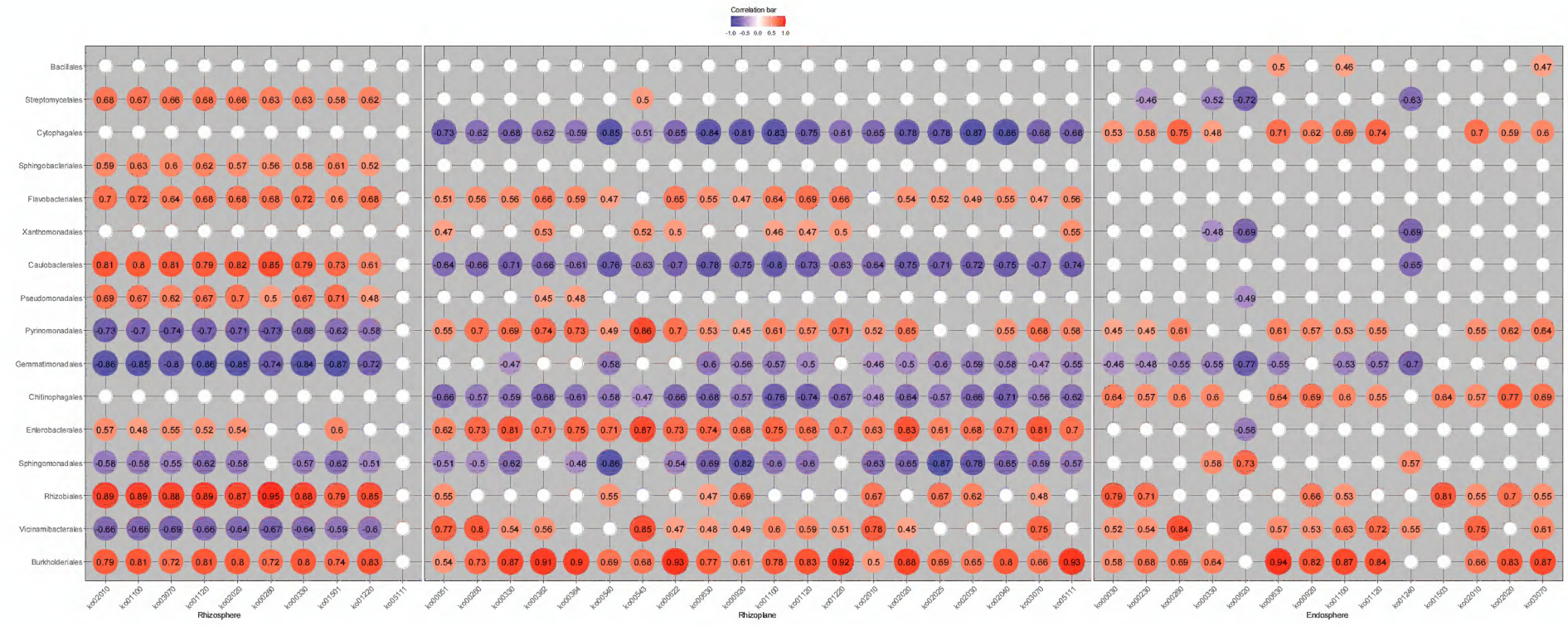


Figure 3. Bar plots the distribution of dominant taxa (**A**) and functional groups (**B**) in bulk soil and rhizocompartments.



Annotations:

ko00260: Glycine, serine and threonine metabolism

ko01100: Metabolic pathways

ko02020: Two-component system

ko00051: Fructose and mannose metabolism

ko00622: Xylene degradation

ko02040: Flagellar assembly

ko01120: Microbial metabolism in diverse environments

ko00330: Arginine and proline metabolism

ko00362: Benzoate degradation

ko00630: Glyoxylate and dicarboxylate metabolism

ko00030: Pentose phosphate pathway

ko02010: ABC transporters

ko05111: Biofilm formation - *Vibrio cholerae*

ko00364: Fluorobenzoate degradation

ko00920: Sulfur metabolism

ko00230: Purine metabolism

ko03070: Bacterial secretion system

ko01220: Degradation of aromatic compounds

ko00540: Lipopolysaccharide biosynthesis

ko02025: Biofilm formation - *Pseudomonas aeruginosa*

ko00620: Pyruvate metabolism

ko01503: Cationic antimicrobial peptide (CAMP) resistance

ko01501: beta-Lactam resistance

ko00543: Exopolysaccharide biosynthesis

ko02030: Bacterial chemotaxis

ko01240: Biosynthesis of cofactors

Figure 4. Heatmap displaying Spearman correlations between dominant taxa and enriched pathways across different rhizocompartments. The white circles, with no correlation values, indicate non-significant correlations ($P > 0.05$).

these factors, respectively. The rhizoplane revealed nitrogen fixation as the primary driver of exchangeable potassium (effK, 9.16), with ureolysis dominating exchangeable nitrogen (effN, 9.76) and nitrate reduction exerting maximal influence on pH (11.75), while effN showed the highest explained variation (19.99%). Functional specialization intensified in the endosphere, where ureolysis (TOC: 13.23; effK: 15.25) and chitinolysis (TOC: 12.59; effN: 7.85) emerged as key players alongside aromatic compound degradation (effN: 12.08; nhN: 12.81), driving substantially higher explained variations for TOC (30.15%), effN (28.69%), and nitrate nitrogen (25.64%). Across compartments, carbon/nitrogen-cycling functions – particularly ureolysis, chitinolysis, Nitrogen fixation, and aromatic degradation – gained prominence, with overall explanatory power for edaphic factors increasing by 8.7–25.64% from rhizosphere to endosphere, reflecting enhanced host-mediated microbial regulation of nutrient dynamics.

Discussion

Cultivar-specific microbial functional diversity and rhizocompartment adaptation

Consistent with previous studies (Patel et al. 2015; Hubbard et al. 2018), the present research underscored the pivotal role of plant genetic factors in shaping the structure and function of microbial communities. Our findings further demonstrate that the assembly of root-associated microbiota exhibits a fine-scale structure across different compartments, a pattern driven primarily by the host cultivar. Notably, as illustrated in Fig. 1, there was a marked increase in the number of differentially abundant KOs from the rhizoplane to the endosphere. Furthermore, notable differences were observed among the cultivars. Specifically, in the rhizoplane, cultivar HY exhibited 880 increased KOs, followed by cultivar JZ with 1071 increased KOs, and cultivar HZ displaying the highest number with 1485 increased KOs. These findings suggest that host genotype selection begins at the rhizoplane (Zhao et al. 2023), highlighting the early impact of genetic factors on microbial community assembly. In addition to the absolute counts of increased KOs, we analyzed the enrichment ratio (increased KOs divided by decreased KOs) across different compartments. Notably, cultivar JZ demonstrated the highest enrichment ratios in both the rhizosphere and endosphere, with values of 5.75 and 1.25, respectively. Cultivar HY exhibited enrichment ratios that fell between those of the other two cultivars across all three compartments, showing a gradual increase from the rhizosphere (0.38) to the rhizoplane (0.81) and finally to the endosphere (0.95). In contrast, cultivar HZ displayed identical enrichment ratios of 0.99 in both the rhizoplane and endosphere. These observations are consistent with the concept of genotype-mediated microbial filtering, as proposed by (Patel et al. 2015), and suggest that genetic signals may regulate microbial entry into the endosphere, as indicated by Bulgarelli et al. (2013).

The variability in pathway enrichment across rhizocompartments underscored plant-driven selective pressures on microbiota. In the rhizosphere, HY showed diverse enrichment, maximizing microbial interactions but raising concerns about functional stability. JZ, conversely, specialized in "Biofilm formation," enhancing nutrient capture under scarcity. On the rhizoplane, all cultivars shared core pathways for root motility and effector delivery, yet exhibited niche-specific adaptations: HZ focused on sulfur and phosphonate metabolism, while JZ prioritized cell cycle

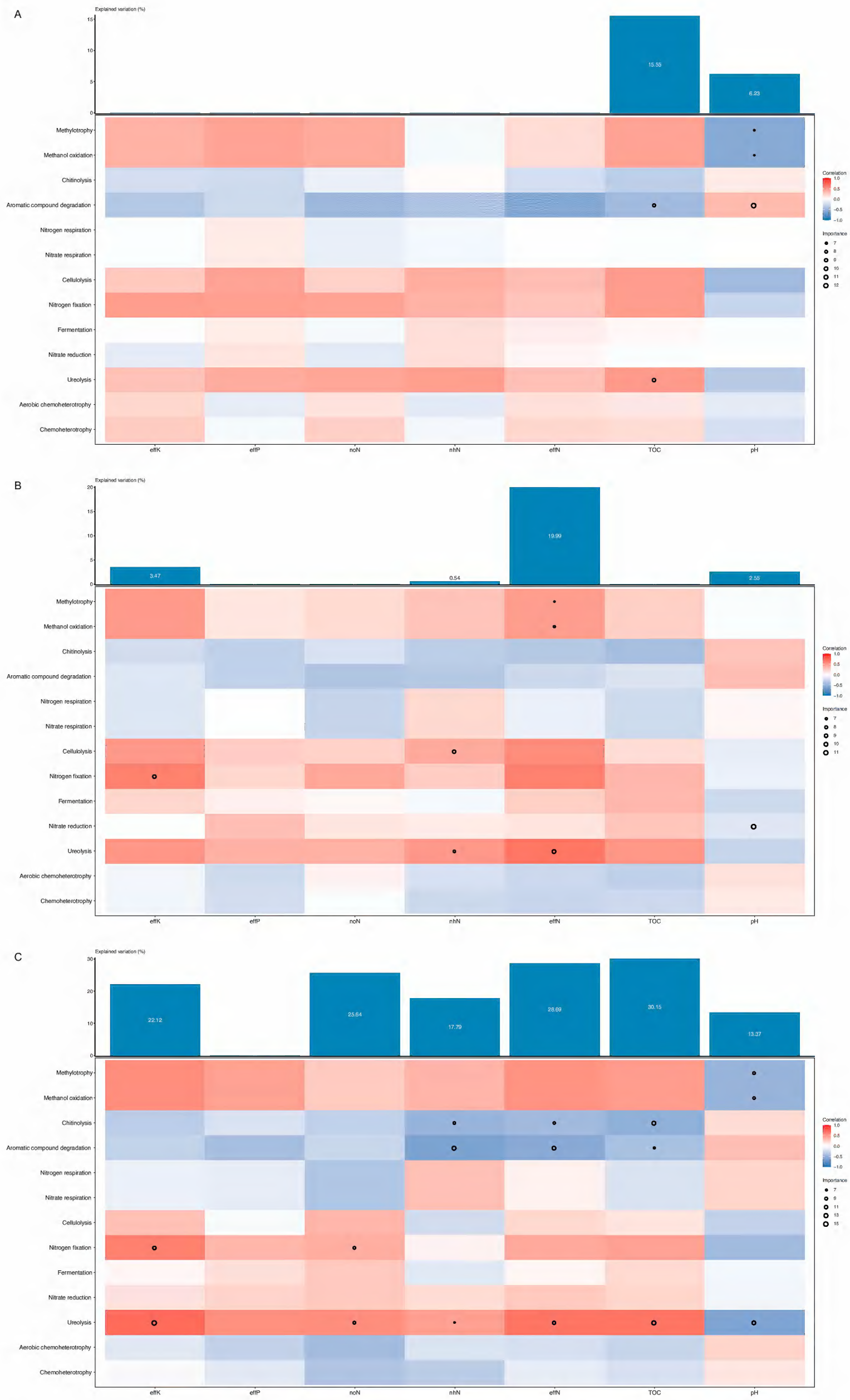


Figure 5. Contributions of functional groups to each edaphic factor during the migration of bacterial communities from bulk soil to the rhizosphere (**A**), rhizoplane (**B**), and endosphere (**C**), respectively, as determined by a random forest regression model. The circle size indicates variable importance, expressed as the percentage increase in mean squared error, while colors represent Spearman correlation coefficients. The bars display the total variation in edaphic factors explained by the functional groups across distinct rhizocompartments.

regulation and pollutant degradation. In the endosphere, pathways diverged sharply, with cultivars displaying unique adaptations like HY's cell wall strengthening and HZ's detoxification prowess. JZ stood out with metabolic autonomy and specialized aromatic compound utilization, aligning with plant-specific needs as proposed by Park et al. (2023)

Distinct functional contributions across rhizocompartments

The analysis of associations between enriched pathways and dominant taxa across rhizocompartments revealed distinct niche-specific microbial functions (Fig. 4). Previous work (Green et al. 2016; Ren et al. 2019; Garcia et al. 2022; Tao et al. 2022) broadly outlined the importance of rhizosphere and endosphere microbiomes in nutrient cycling and plant health, but detailed taxonomic-functional linkages and compartment-specific profiles remained underexplored. Our results elucidated these associations with a new level of granularity, revealing both cultivar-dependent and compartment-specific microbial interactions. In the rhizosphere, taxa such as Rhizobiales were strongly correlated with nutrient cycling pathways, notably glycine, serine, and threonine metabolism (ko00260, $r = 0.95$), a finding consistent with prior studies that emphasize the role of Rhizobiales in nitrogen fixation and amino acid metabolism (Ao et al. 2021; Kullapanich et al. 2021; Zhukova et al. 2022). However, the pronounced negative correlations observed for Gemmatimonadales and Pyrinomonadales with critical metabolic pathways like ABC transporters (ko02010, $r = -0.86$) highlighted niche competition and metabolic antagonism, underscoring functional differentiation within the rhizosphere.

In the rhizoplane, the dominance of Burkholderiales in pathways like benzoate degradation (ko00362, $r = 0.93$) and xylene degradation (ko00622, $r = 0.93$) suggested a pivotal role in pollutant breakdown and environmental resilience, extending findings from Edwards et al. (Edwards et al. 2015), who noted Burkholderiales' adaptability to environmental stresses. Additionally, correlations with microbial metabolism in diverse environments (ko01120, $r = 0.83$) emphasized their versatility. Sphingomonadales, however, showed negative correlations with sulfur metabolism (ko00920, $r = -0.82$), suggesting specialization in less sulfur-dependent niches. Zhao et al. (2023) demonstrated that the rhizoplane played a pivotal gating role in restricting microbial penetration into the endosphere. These findings further emphasized that, although metabolic diversity was preserved across rhizocompartments, the rhizoplane functioned as a transitional zone, characterized by heightened functional specialization that underscored its critical role in microbial selection and environmental adaptation.

The endosphere presented the most host-integrated microbial metabolic profiles. Functional groups, particularly Burkholderiales and Rhizobiales, exhibited strong correlations with pathways central to oxidative stress management (e.g., sulfur metabolism, ko00920, $r = 0.82$) and adaptive signaling (e.g., two-component systems, ko02020, $r = 0.83$). These results extended Wicaksono et al.'s (Wicaksono et al. 2022) viewpoint of host-imposed selection pressures by providing empirical evidence for compartmentalized metabolic prioritization in endophytic communities. Negative correlations for taxa such as Vicinamibacterales and Pyrinomonadales with biosynthetic pathways (e.g., biosynthesis of cofactors, ko01240, $r = -0.70$) reflected resource partitioning driven by competitive exclusion.

These findings supported a hierarchical model of plant-associated microbiomes, where rhizosphere microbiota drive general metabolic functions, rhizoplane communities specialize in stress-related pathways, and endosphere microbiota integrate metabolic roles with host physiology. This compartmentalization refined previous broad categorizations of plant microbiota and suggested opportunities for precision microbiome engineering targeting specific plant compartments. While our results demonstrate clear compartmentalization of microbial communities, the underlying mechanisms warrant further investigation. We hypothesize that cultivar genetic variation may influence root exudate composition (including sugars, amino acids, and organic acids), which could subsequently shape microbial assembly by selecting taxa capable of metabolizing these compounds. However, this proposed genotype-exudate-microbiome relationship remains speculative and requires direct experimental validation in future studies focusing on the dynamic interactions between sorghum genetics, root exudation patterns, and microbiome assembly.

Contributions of functional groups to edaphic dynamics

The variations of functional groups among cultivars across rhizocompartments (Fig. 3B) extended prior research (Brescia et al. 2023; Kuźniar et al. 2023) by illustrating that while chemoheterotrophy and aerobic chemoheterotrophy were core functions of root-associated microbiome, considerable cultivar-dependent variations existed. The higher abundance of chemoheterotrophs in HZ's rhizosphere relative to JZ suggested selective pressures exerted by root exudates, implying genotype-specific impacts on rhizosphere microbiota. Additionally, the pronounced enrichment of "Nitrate reduction" in JZ's endosphere provided novel insights into genotype-driven functional adaptations, potentially linked to enhanced nitrogen cycling. The elevated "Ureolysis" in HY's rhizoplane highlighted compartment-specific microbial niches, underscoring root compartmentalization's influence on functional group distribution. Moreover, the higher relative abundance of "Human-associated" and "Animal parasites or symbionts" in the HY endosphere suggested the presence of microbes typically found in diverse environments, including soil and host-associated niches. This pattern not only reflects a more diverse microbial assemblage but may also indicate broader ecological interactions be-tween the host plant and its surrounding biota.These results collectively supported a universal yet cultivar-mediated framework for microbial

community assembly, offering critical implications for crop microbiome optimization in sustainable agriculture.

Our previous research (Zhao et al. 2023) demonstrated that edaphic factors displayed a gradient distribution from bulk soil to the rhizosphere, correlating with microbial succession from bulk soil to various rhizocompartments. While prior studies primarily concentrated on the overall role of microbial communities in soil health, they often neglected the influence of specific functional groups on edaphic factors during their succession from bulk soil to different rhizocompartments. This study addressed this oversight by exploring how functional groups affect key edaphic factors critical for nutrient cycling and soil fertility.

The rhizocompartment-specific gradients of functional group contributions revealed distinct microbial roles in soil nutrient cycling and environmental adaptation (Fig. 5). In the rhizosphere, functional groups like aromatic compound degradation and ureolysis exhibited high contributions to TOC (importance scores of 9.31 and 8.27, respectively). These results aligned with prior findings on the role of ureolysis in nitrogen cycling (Wang et al. 2023; Yuan et al. 2023) but expanded on them by quantifying its impact on TOC, emphasizing the moderate contribution of microbial activities to soil carbon cycling. The rhizoplane displayed greater functional diversity, with ureolysis and cellulolysis significantly influencing effN (9.76 and 8.89, respectively). This diversity highlights the rhizoplane's role as a dynamic interface where microbial metabolism transitions from general to more specialized nutrient cycling. Notably, nitrate reduction's pronounced effect on pH regulation (11.75) underscored a critical microbial-mediated mechanism for maintaining soil homeostasis, corroborating Wang et al.'s (Wang et al. 2024) findings on nitrogen control of soil pH. The endosphere exhibited a starkly different functional landscape, with specialized contributions from groups like chitinolysis and aromatic compound degradation to TOC and effN. The dominance of ureolysis (15.25 for effK) and aromatic compound degradation (12.81 for nhN) demonstrated a focused microbial adaptation to host-imposed selective pressures. These results align with recent hypotheses regarding host-microbe co-evolution but go further by demonstrating the direct quantitative impact of these functions on nutrient availability, with explained variations surpassing 30% for TOC and effN—far exceeding those observed in other rhizocompartments.

The endosphere revealed the most pronounced microbial specialization, with ureolysis, chitinolysis, and aromatic compound degradation playing dominant roles in modulating TOC, effN, and nhN. These findings expanded upon the work of Li and Gao (2023), who proposed that host-driven selection shapes endosphere microbiota. The higher explained variance for TOC (30.15%) and effN (28.69%) in this compartment underscores the selective optimization of microbial activities that enhance plant nutrient acquisition and organic matter decomposition, likely mediated by host signaling and symbiosis.

In summary, as microbial communities transitioned from the rhizosphere to the endosphere, the importance of functional groups in carbon and nitrogen cycling progressively increased. This gradient reflects a shift from externally driven to host-mediated microbial processes, as plants impose stronger selection pressures on endophytic communities to optimize nutrient acquisition and environmental stress responses. These findings underscore the importance of integrating functional group analyses with metagenomic and transcriptomic approaches in future studies to further unravel temporal dynamics and mechanistic underpinnings of plant-microbe-soil systems.

Conclusion

This study drew several conclusions: (1) Sorghum cultivar considerably influenced microbial community functions, with notable compartment-specific stratification observed across different cultivars; JZ demonstrated the highest enrichment of beneficial microbial functions in the endosphere, enhancing biofilm formation and nutrient transport; (2) The variability in pathway enrichment highlighted selective pressures exerted by plants on their associated microbiota, where JZ's specialized pathways aimed at nutrient capture contrasted with HY's broader but less efficient functional profile; (3) Distinct microbial taxa like Rhizobiales and Burkholderiales showed strong correlations with crucial metabolic pathways, indicating their roles in nutrient cycling and pollutant degradation across rhizocompartments; (4) The compartmental analysis illuminated how specific plant genotypes optimized microbial interactions through tailored metabolic pathways, with JZ facilitating partnerships under stress and HZ enhancing dynamic colonization; (5) Functional contributions from microbial groups revealed a gradient of influence from rhizosphere to endosphere, highlighting the rising specialization and importance of microbial roles in modulating key soil factors such as TOC, nhN and effN. This study expanded upon existing literature by emphasizing cultivar-specific impacts on microbial dynamics and opened up avenues for future research focused on the genetic and biochemical interactions driving these relationships. Integrating these insights into microbiome engineering could significantly enhance crop productivity and resilience in diverse agricultural contexts.

Author Contributions

Conceptualization, L.Z. and X.Y.W.; methodology, K.Y.Z and H.L.Y.; validation, F.Y.W; investigation, K.Y.Z, H.L.Y. and L.J.; resources, X.Y.W.; writing—original draft preparation, L.Z.; writing—review and editing, X.Y.W.; supervision, G.D.G, Z.M. and Y.Z.; funding acquisition, L.Z. and X.Y.W.

Data availability statement

The 16S rRNA gene amplicon sequences associated with this study have been deposited in the NCBI SRA under accession: PRJNA945624.

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Supplementary material

Supplementary material 1
appendix S1. Field experiment and sample collection; table S1. The quantified values of edaphic factors for bulk soil and rhizosphere soil from the samples analyzed in this study (docx)
Link: https://doi.org/10.3897/ejfa.2025.171979.suppl1